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Which molecular targets are most relevant to general anaesthesia?

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Abstract

1. In view of the large number of possible molecular targets of general anaesthetics, it is necessary to have some criteria for judging which targets are important for producing general anaesthesia and which are probably not. 2. We consider in detail two criteria: sensitivity to clinically relevant concentrations of anaesthetics and stereoselectivity to anaesthetic optical isomers. 3. The targets which currently emerge as most important belong to an anaesthetic-sensitive superfamily of genetically related fast neurotransmitter-gated receptor channels present at central synapses.

Keywords: Anaesthetic concentrations; $GABA_A$ receptors; Ligand-gated channels; Optical isomers; Stereoselectivity

1. Introduction

One of the major problems in anaesthesia research is choosing which systems to study. General anaesthetics can affect an almost infinite variety of molecular systems, especially if one uses free aqueous anaesthetic concentrations that are substantially greater than those that are needed to produce mammalian general anaesthesia. It therefore follows that a candidate system should be significantly perturbed by clinically relevant concentrations of general anaesthetics, and, other things being equal, preference should be given to those putative targets that are most sensitive. However, other things are not always equal, and additional criteria are needed. A criterion we have proposed is based upon the fact that general anaesthesia is stereoselective for a number of commonly used agents (Franks and Lieb, 1994). If there are only a relatively few important primary targets, then the rank order and degree of stereoselectivity found with anaesthetic optical

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isomers for general anaesthesia might be expected to be mimicked by in vitro studies on these primary targets. Another criterion, which assumes a single (unitary) site of general anaesthetic action, makes use of anaesthetic-like compounds that are devoid of general anaesthetic activity in animals at concentrations predicted to be anaesthetic by the Meyer–Overton rule (Koblin et al., 1994). If one of these so-called 'nonanaesthetics' produce significant in vitro effects on a putative target, then, according to this criterion, it can be ruled out as an important target in general anaesthesia.

2. Anaesthetic sensitivity

2.1. Anaesthetic concentrations

We will first address the problem of 'clinically relevant' anaesthetic concentrations. It is commonplace for workers in our field to assume very high values for such concentrations and to therefore ascribe erroneously high anaesthetic sensitivities to putative targets. The reasons for this are different for volatile and intravenous agents, and we shall consider each type of anaesthetic in turn.

Volatile agents are administered to mammals in the gas phase at body temperature ($\sim$37°C), while most in vitro experiments are (for experimental convenience) carried out at room temperature (20–25°C). However, gas-phase potencies are usually very temperature-dependent, increasing markedly with decreasing temperatures. We have argued (Franks and Lieb, 1996a) that this is largely due to the temperature dependence of anaesthetic partitioning between gas and condensed phases, so that the common procedure of using gas-phase EC_{50} concentrations for room temperature experiments can result in overdosing the in vitro preparation several-fold. We have shown that aqueous-phase EC_{50} concentrations for general anaesthesia with volatile agents are much less dependent on temperature and can, to a good approximation, be used for in vitro experiments at either body or room temperature (Franks and Lieb, 1996a). A list of our recommended free aqueous EC_{50} (MAC) concentrations for mammalian general anaesthesia appears in Table 1. It must be stressed that these concentrations are upper estimates, using as the anaesthetic endpoint a lack of a purposeful response to a painful stimulus. Were we to use loss of a response to a verbal command (MAC-Awake) in patients (Marshall and Longnecker, 1996) or loss of righting response in rodents (Deady et al., 1981; Kissin et al., 1983) as the anaesthetic endpoint, the EC_{50} values would be substantially smaller (typically 2- to 4-fold).

For intravenous agents, on the other hand, excessive in vitro concentrations are often used due to a failure to take proper account of the often complex pharmacokinetic factors (binding to plasma proteins, redistribution, metabolism, permeability, etc.) operative during the induction of, and recovery from, general anaesthesia. Concerning binding to plasma proteins, it is surprisingly often ignored that it is the free, and not the total, plasma or blood concentration which is relevant for in vitro experiments. For example, $\sim$98% of plasma propofol is bound to plasma proteins. If this is not corrected for, and it often is not, a free aqueous concentration of propofol may be used which is some 50-fold in excess of that needed for general anaesthesia. Another source of confusion is the use of the transient peak (blood or plasma) concentrations following a bolus injection. While of obvious clinical importance, it is almost impossible to relate this concentration to that relevant or appropriate for in vitro experiments. We feel the only safe practice is to

Table 1
Mammalian MAC values for volatile anaesthetics expressed as free aqueous concentrations (C_{aq}) in saline

Agent	C_{aq} (mM)
Halothane	0.23
Isoflurane	0.28
Enflurane	0.59
Desflurane	0.56
Sevoflurane	0.33

Values of C_{aq} were calculated using literature values of anaesthetic partial pressures together with values of Bunsen or Ostwald saline/gas partition coefficients at 37°C. Although all data are for 37°C, the C_{aq} values are reasonable approximations (to within $\sim$25%) for use at room temperature in in vitro experiments. See Franks and Lieb (1996a) for further details.

Table 2
Mammalian 'MAC equivalent' values for intravenous anaesthetics expressed as free aqueous concentrations (C_{aq}) in water

Agent	C_{aq} (μM)
Thiopental	25
Pentobarbital	50
Propofol	1.5

Values of C_{aq} are our best estimates for the free aqueous concentrations required to half-inhibit a response to a painful stimulus in mammals. See Franks and Lieb (1996a) and Violet et al. (1997) for further details.

use the quasi steady-state concentrations of intravenous agents needed to maintain general anaesthesia. Finally, as with volatile agents, the concentrations of intravenous anaesthetics needed to produce general anaesthesia depend on the endpoints used, being considerably lower for loss of appropriate responses to painless stimuli. For example, the steady-state free aqueous thiopentone concentration for maintaining general anaesthesia in rats can be calculated (Franks and Lieb, 1994) from the data of Gustafsson et al. (1996) to be 42 μM for an intubation response, 24 μM for a tail-clamp response, and only 10 μM for an unprovoked righting response. In Table 2 we have listed free aqueous EC_{50} concentrations corresponding to a painful stimulus.

2.2. Relatively insensitive neuronal targets

Until recently, it was thought that lipid bilayers were the primary sites of general anaesthetic action. However, structural and dynamic studies have now shown that effects of clinical concentrations of anaesthetics on lipid bilayer models of neuronal plasma membranes are so small that in almost all cases they can be mimicked by a temperature change of ≤ 1°C (Franks and Lieb, 1982). This, together with the demonstration (see below) that optical isomers of isoflurane can act stereoselectively on some ion channels, but not on lipid bilayers, has led to the abandonment of the lipid hypothesis by most workers in the field (but see other contributors in this volume).

It has long been known that axonal conduction is less sensitive to general anaesthetics than is synaptic transmission (Pocock and Richards, 1993), and the voltage-gated Na^+ and K^+ channels which produce action potentials are usually thought to be very insensitive to general anaesthetics (Elliott and Haydon, 1989; Franks and Lieb, 1991b), though Rehberg et al. (1996) have shown that the Na^+ channels can be sensitive under certain conditions. Similarly, voltage-gated fast transient K^+ currents ('I_A') are quite insensitive to volatile agents (Franks and Lieb, 1991a,b). Voltage-gated Ca^{2+} channels are probably the most anaesthetic-sensitive of all voltage-gated channels (Franks and Lieb, 1993, 1994) and are, in addition, plausible presynaptic targets. However, the P-type channel in rat CNS neurons (the channel subtype thought to be the most important for central synaptic transmission) is only minimally inhibited ($\leq 10\%$) by clinically relevant concentrations of commonly used volatile and intravenous general anaesthetics (Fig. 1 and Hall et al., 1994a). Similarly small degrees of inhibition have generally been found for other voltage-gated Ca^{2+} channel subtypes by other workers (Franks and Lieb, 1993, 1994). Overall, it currently appears that most voltage-gated cation channels are relatively insensitive to general anaesthetics.

Compared to anaesthetic studies on lipid bilayers and ion channels, far less work has been carried out on second messenger systems. We do not intend to review this potentially important area here. In brief, the current view is that many of these systems are relatively insensitive to clinically relevant concentrations of general anaesthetics, and there is as yet little solid evidence for the direct involvement of second messengers in the production of general anaesthesia (Franks and Lieb, 1994). This view may well change as more results accumulate.

2.3. An anaesthetic-sensitive superfamily

There is a superfamily (Fig. 2) of genetically and structurally related receptor channels that are directly gated by neurotransmitters at central synapses and are sensitive to modulation by many general anaesthetics (Franks and Lieb, 1996b). Mammalian members of this superfamily include the excitatory nicotinic acetylcholine and 5-HT_3

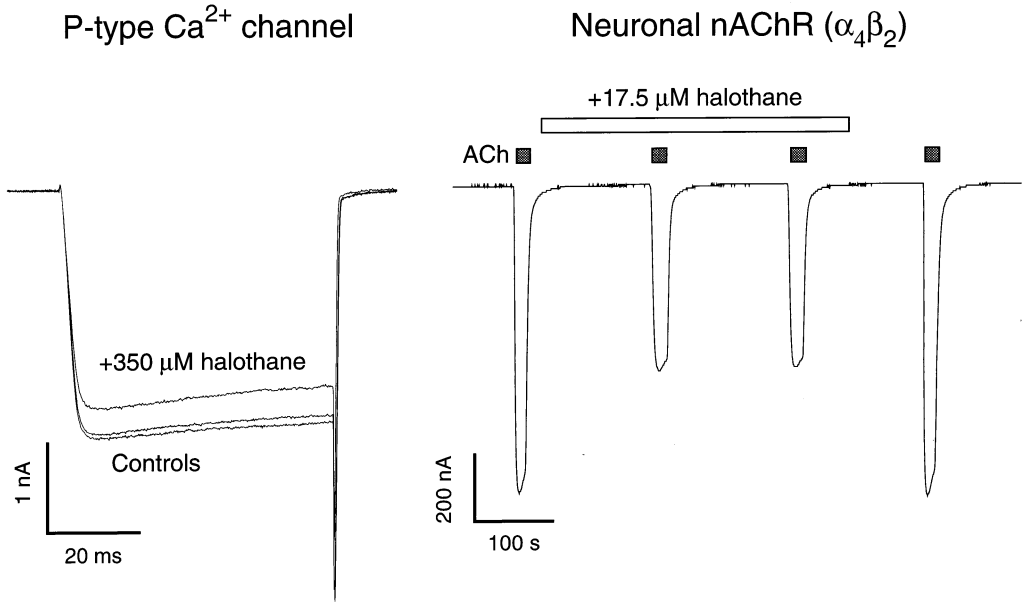

Fig. 1. Some general anaesthetic targets are very much more sensitive than others. P-type calcium channels in rat cerebellar Purkinje neurons are inhibited by only ~10% by 350 μM halothane (Hall et al., 1994a), while a 20-fold lower concentration nearly half-inhibits a rat neuronal nicotinic acetylcholine receptor expressed in *Xenopus* oocytes (Violet et al., 1997).

receptors, and the inhibitory $GABA_A$ and glycine receptors (Unwin, 1993; Ortells and Lunt, 1995). The receptor channels for L-glutamate, the major excitatory neurotransmitter in the mammalian CNS, do not belong to this superfamily (Dani and Mayer, 1995) and are insensitive to many anaesthetics, especially volatile agents (Franks and Lieb, 1994; Perouansky et al., 1995).

In comparison to the small effects of anaesthetics at clinically relevant concentrations on voltage-gated channels, effects on members of the anaesthetic-sensitive superfamily can be very large (Fig. 1). For example, at about 1 MAC isoflurane the mammalian P-type voltage-gated Ca^{2+} channel is inhibited by only 8% (Hall et al., 1994a), whereas the mammalian neuronal nicotinic acetylcholine receptor channel is inhibited by over 70% (Violet et al., 1997; Flood et al., 1997) and the mammalian $GABA_A$ channel potentiated by about 100% (Hall et al., 1994b).

The mammalian neuronal nicotinic acetylcholine receptor channel (neuronal nAChR) has recently been found to be much more sensitive to general anaesthetics than the much-studied muscle nAChR, when both subtypes were expressed in *Xenopus* oocytes (Violet et al., 1997). The anaesthetic IC_{50} concentrations for inhibiting the neuronal nAChR were 10- to 35-fold less than those for the muscle nAChR, for halothane, isoflurane, sevoflurane and propofol. In fact, the neuronal nAChR was supersensitive to volatile agents (Fig. 1). For example, the IC_{50} for isoflurane inhibition of the most prevalent heteromeric form of the receptor in the brain ($\alpha_4\beta_2$) was only 34 μM, which can be compared with a free aqueous MAC concentration of 270 μM for surgical general anaesthesia. On the other hand, this supersensitivity did not extend to the intravenous anaesthetic propofol, whose IC_{50} (4.5 μM) was 3-fold larger than its EC_{50} (Table 2) for general anaesthesia. Volatile agents have also been found to be on average more sensitive than other agents at inhibiting nAChR chloride channels in molluscan neurons (McKenzie et al., 1995), though supersensitivity was not observed. In this latter study, IC_{50} concentrations for 30 anaesthetics inhibiting the neuronal nAChR were found to reasonably well match EC_{50} concentrations for tadpole general

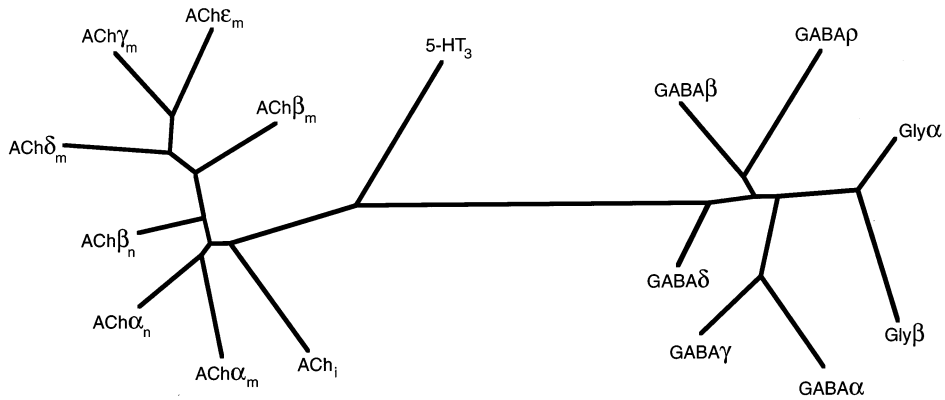

Fig. 2. The anaesthetic-sensitive superfamily of fast, neurotransmitter-gated receptor channels. This phylogenetic tree is a simplified version of one given by Ortells and Lunt (1995). The distance between any two subunits in the diagram is a measure of the similarity of their DNA base sequences (Downie et al., 1996).

anaesthesia. It remains to be seen if supersensitivity to volatile agents is a property of nAChRs in mammalian central neurons.

Whereas the excitatory mammalian neuronal nAChR is inhibited by general anaesthetics, the inhibitory $GABA_A$ and glycine receptor chloride channels are almost invariably potentiated. The $GABA_A$ receptor is sensitive to a remarkably diverse range of anaesthetics, including volatile agents, alcohols, anaesthetic steroids and barbiturates (Olsen et al., 1986; Wakamori et al., 1991; Franks and Lieb, 1994; Hall et al., 1994b; Wittmer et al., 1996). Indeed, there appear to be only a few general anaesthetics (such as ketamine) that do not potentiate the most common forms of the $GABA_A$ receptor at clinically relevant concentrations. Interestingly, certain minor $GABA_A$ subtypes containing δ or ε subunits, appear to be insensitive to some intravenous agents (Zhu et al., 1996; Davies et al., 1997). The closely related glycine receptor has been studied much less but, like the $GABA_A$ receptor, appears to be potentiated by most general anaesthetics, with the exception of etomidate and ketamine (Downie et al., 1996; Mascia et al., 1996). Anaesthetic potentiation of the closely related $GABA_A$ and glycine receptors involves an increase in the apparent affinity to neurotransmitter agonist, which at the high agonist levels thought to be present at synapses would translate to a prolongation of inhibitory postsynaptic potentials (Franks and Lieb, 1994). The molecular mechanisms underlying these allosteric effects are beginning to be unravelled using the techniques of molecular genetics (Mihic et al., 1997).

The excitatory 5-HT_3 receptor channel, the final member of the mammalian anaesthetic-sensitive superfamily, occupies a position intermediate to the inhibitory $GABA_A$ and glycine receptors (which are potentiated by general anaesthetics) and the excitatory nAChRs (which are inhibited by anaesthetics). The 5-HT_3 receptor (Machu and Harris, 1994; Jenkins et al., 1996) is both potentiated by some anaesthetics (volatile agents, low concentrations of lower *n*-alcohols) and inhibited by others (high concentrations of thiopentone and lower *n*-alcohols, reasonable concentrations of higher *n*-alcohols). The existence of both potentiating and inhibiting anaesthetic sites on the 5-HT_3 receptor may be related to its putative status as the most primitive member of the anaesthetic-sensitive superfamily (Ortells and Lunt, 1995).

3. Anaesthetic optical isomers

Sensitivity to clinically relevant concentrations of general anaesthetics is certainly a requirement for a putative target site, but it is not the only criterion one can use. Another test is based upon the often unappreciated fact that general anaesthesia in mammals is stereoselective for many

optically active agents such as isoflurane, etomidate, ketamine, barbiturates and neurosteroids (Franks and Lieb, 1994). The degree of stereoselectivity varies considerably in mammals, being a factor of 1.5 or less for isoflurane, about 2-fold for barbiturates, 2- to 4-fold for ketamine, and up to a factor of 10 or more for neurosteroids (Franks and Lieb, 1994; Wittmer et al., 1996; Tomlin et al., 1998). If there are only a few major targets for a given agent, then the rank order and degree of stereoselectivity found for general anaesthesia with that agent should be mimicked by in vitro studies on the putative target(s) for that agent. For example, the stereoselective effects of ketamine enantiomers on the NMDA glutamate receptor channel closely mirror those in whole animals (Franks and Lieb, 1994), consistent with this receptor being a major target for ketamine general anaesthesia. On the other hand, isoflurane partitions non-stereoselectively into lipid bilayers (Dickinson et al., 1994), consistent with membrane lipids not being a major target for anaesthesia with volatile agents. These examples illustrate a fundamental advantage of the use of optical isomers over nonanaesthetics (Section 1) to reveal putative targets, since the former (but not the latter) method does not assume a unitary site of action and the agents being tested are exactly the anaesthetics of interest, rather than chemically related analogues.

Experiments with the isoflurane enantiomers have shown that S(+) isoflurane is slightly (by a factor of 1.5 or less) more potent than R(−) isoflurane for rat general anaesthesia (Lysko et al., 1994; Eger et al., 1997) but considerably more effective (about 2-fold at MAC) at potentiating the rat $GABA_A$ receptor (Hall et al., 1994b and Fig. 3). Similar results were obtained on $GABA_A$ receptor-mediated IPSCs at inhibitory synapses in cultured rat neurons (Jones and Harrison, 1993). These large in vitro effects suggest that the $GABA_A$ receptor is one of the, but certainly not the only, principal sites of action for volatile agents. Similarly large stereoselectivities have been reported for a volatile anaesthetic-activated K^+ current ('I_{KAn}') and a neuronal nAChR in molluscan neurons (Franks and Lieb, 1991a), though isoflurane stereoselectivity for molluscan general anaesthesia has yet to be investigated. Not all targets exhibit stereoselectivity for isoflurane. For example, isoflurane inhibition of a fast transient K^+ current ('I_A') in molluscan neurons (Franks and Lieb, 1991a), inhibition of isradipine binding to mouse L-type voltage-gated Ca^{2+} channels (Moody et al., 1994), and potentiation of mammalian glycine receptor channels (Downie et al., 1996) show little if any stereoselectivity.

Etomidate appears to be very specific for $GABA_A$ receptor channels (Tomlin et al., 1998). Consistent with this, we have found that R(+) etomidate is some 5- to 10-fold more potent than its S(−) enantiomer at potentiating $GABA_A$ receptors. (Animal potency data for mammals is lacking, but in tadpoles we found the same rank order and a similar potency ratio for general anaesthesia produced by the etomidate enantiomers.) As with isoflurane (Franks and Lieb, 1991a), no stereoselectivity was found for etomidate interacting with lipid bilayers (Tomlin et al., 1998). Neuroactive steroids are also thought to selectively potentiate $GABA_A$ receptors, and a recent study by Wittmer et al. (1996) is consistent with this view. Enantiomers of two neurosteroids were each found to exhibit the same rank orders of effectiveness for tadpole general anaesthesia

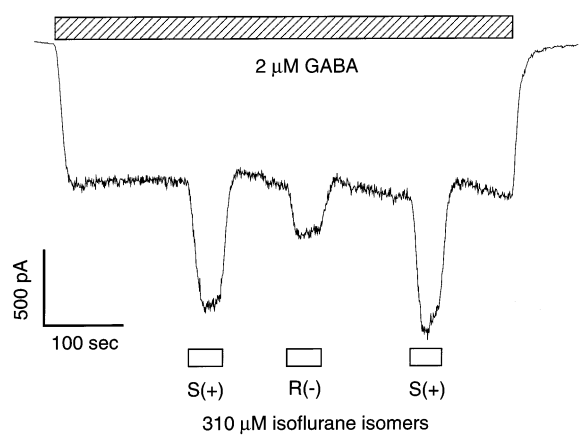

Fig. 3. Stereoselective actions of isoflurane enantiomers acting on the $GABA_A$ receptor. The S(+) enantiomer of isoflurane is more effective than the R(−) enantiomer at potentiating GABA-induced currents in rat cerebellar Purkinje neurons, in line with their relative anaesthetic potencies in animals (Hall et al., 1994b).

and for potentiating $GABA_A$ receptor channels in rat CNS neurons. One of the enantiomeric pairs was also tested for mouse general anaesthesia, with the same result as with tadpoles. The chiral effects were large both in vivo and in vitro, in support of a major role for $GABA_A$ receptors in the general anaesthesia produced by neuroactive steroids. Finally, a number of barbiturates have been found to interact stereoselectively with $GABA_A$ receptors with in vitro binding assays (Olsen et al., 1986) and with whole animals in vivo (Franks and Lieb, 1994), but reliable modern studies in which the effects of barbiturate enantiomers on $GABA_A$-mediated currents have been compared with their effects as general anaesthetics in mammals are lacking.

Overall, the use of anaesthetic optical isomers as a criterion for judging which molecular targets are important in general anaesthesia has only begun to be exploited, largely due to the commercial unavailability of these chiral compounds in sufficient purity. What work has been carried out has focused largely on $GABA_A$ receptor channels, and present results are consistent with an important role for these ubiquitous receptors in general anaesthesia. Future studies on other putative targets will lead not only to the identification of other possible targets but also to a clarification of whether or not in the clinical context it is advantageous to consider administering anaesthetic enantiomers in place of the racemic mixtures in current use.

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